WILD ANIMAL MORTALITY INVESTIGATION: SOUTHERN RESIDENT KILLER WHALE L-112 FINAL REPORT [Draft 02-24-14]

Authors and Affiliations:

Debbie Duffield, Department of Biology, Portland State University, Portland, OR 97207; duffieldd@pdx.edu
Joseph K. Gaydos, The SeaDoc Society, UC Davis Wildlife Health Center, Orcas Island Office, 942 Deer Harbor R., Eastsound, WA 98245; jkgaydos@ucdavis.edu
Stephen Raverty, Animal Health Center, 1767 Angus Campbell Road, Abbotsford, BC, Canada V3G 2M3; stephen.raverty@gov.bc.ca
Kristin Wilkinson, NOAA Fisheries, Protected Resources Division, 7600 Sandpoint Way, NE, Seattle, WA 98115; kristin.wilkinson@noaa.gov
Brent Norberg, NOAA Fisheries, Protected Resources Division, 7600 Sandpoint Way. NE, Seattle, WA 98115; brent.norberg@noaa.gov
Lynne Barre, NOAA Fisheries, Protected Resources Division, 7600 Sandpoint Way, NE, Seattle, WA 98115; lynne.barre@noaa.gov
Brad Hanson, NOAA/NMFS/ Northwest Fisheries Science Center, 2725 Montlake Blvd. E, Seattle, WA 98112; brad.hanson@noaa.gov
Peggy Foreman, NOAA Fisheries, NWR Communications Division, 7600 Sandpoint, Way NE, Seattle, WA 98115; peggy.foreman@noaa.gov
Amy Traxler, The Whale Museum, P.O. Box 945, Friday Harbor, WA 98250; eric@whalemuseum.org
Dyanna Lambourn, Marine Mammal Research Biologist, Washington Department of Fish and Wildlife, 7801 Phillips Rd. SW., Lakewood, WA 98498; dyanna.lambourn@dfw.wa.gov
Jessie Huggins, Cascadia Research Collective, 218 ½ W 4th Ave., Olympia, WA 98501; jhuggins@cascadiaresearch.org
John Calambokidis, Cascadia Research Collective, 218 ½ W 4th Ave., Olympia, WA 98501; calambokidis@cascadiaresearch.org
Tori McKlveen, Diplomate, American College of Veterinary Radiology, VCA Veterinary Specialty Center of Seattle, 20115 44th Ave West, Lynnwood, WA 98036; tori.mcklveen@vcahospitals.com
Sophie Dennison, Animal Internal Medicine and Specialty Services, 1333 9th Ave., San Francisco, CA 94122; vetradiologist@hotmail.com
Hans Brubaker, NOAA Fisheries, Protected Resources Division, 7600 Sandpoint Way. NE, Seattle, WA 98115; hans.brubaker@noaa.gov

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EXECUTIVE SUMMARY

On February 11, 2012 a 3.75-meter total length juvenile female Southern Resident killer whale (SRKW), identified as L-112, stranded just north of the town of Long Beach, Washington. The following day the carcass was taken to a secure location at Cape Disappointment State Park for a full necropsy by biologists and volunteers from the Northwest Marine Mammal Stranding Network (Network). This report reviews the data from the necropsy, histopathology, and computed tomography (CT) examinations, as well as results from ancillary diagnostic tests. Available information on environmental data and human activities were compiled and evaluated to assess possible contributions to the loss of the animal.

Gross examination revealed the whale was moderately decomposed but in good nutritional condition. On the back of the head and neck was extensive subcutaneous bruising, which extended deep into the adjoining musculature and tracked along the hypodermis to the throat region. On the right side, bruising extended to the anterior insertion of the pectoral fin. Initial estimates of time of death were 4 to 10 days prior to the stranding.

A detailed dissection and CT scan of the head confirmed the gross observations of hemorrhage and revealed extensive gas and fluid accumulation. No skull fractures were noted. No fractures or dislocations of the bones of the middle and inner ear were seen. A CT scan of the cervical vertebrae was also performed. There was incomplete fusion of the dorsal aspect of the C7 vertebral body that was attributed to a congenital anomaly and was not considered clinically significant. No broken bones were found on preparation of the skeleton. However, there was a long linear crack on upper right jaw tooth #13, and based on its gross appearance and orientation, it was not considered a consequence of terminal trauma.

Microscopic review of the sampled tissues was hindered by the extent of post-mortem decomposition. Based on the degree of autolysis and mass spectrophotometry results of sampled tissue gases (Woods Hole Oceanographic Institute, Massachusetts), the gas accumulation (emphysema) throughout the carcass was attributed to putrefaction rather than gas bubble-type disease as previously reported in stranded beaked whales exposed to naval sonar or common dolphins with submerged blast injury. On dissection of the inner ear from the skull, a parasite (nematode) infection was observed around the right bulla, with associated inflammation detected microscopically. There was mild, chronic inflammation of the gastrointestinal tract and low to intermediate grade accumulation of scar tissue (fibrosis) in the heart, liver, and kidney. None of these were sufficiently severe to be assigned as a specific cause of death. Extensive molecular (polymerase chain reaction or PCR), bacteriology, virology, CT scans, and toxicology tests were undertaken and no significant disease agents or pathologic entities were identified. The light bacterial growth recovered from harvested tissues was attributed to post-mortem tissue invasion and overgrowth. Results from heavy metal and trace mineral analyses of the liver and kidney were largely within normal reference ranges and persistent organic pollutant levels in blubber were within anticipated levels for SRKWs.

The nematodes in the right bulla were identified as *Crassicauda* sp. Although ear infections with *Crassicauda* are more commonly recognized in smaller stranded cetaceans, detection in this animal is not unusual and would likely not have interfered with normal inner ear function (deafness or vestibular dysfunction).

Stomach contents analysis revealed six fish eye lenses and nematode fragments identified as *Anisakis* sp. cf. *A. simplex*. PCR analysis of feces from L-112 detected Chinook salmon and halibut, consistent with dietary preferences of SRKWs.

Data collected by autonomous passive acoustic recorders deployed off the coasts of Washington, Oregon, and California were reviewed. These devices monitor killer whales by recording their pod-specific calls and identified the presence and movements of L pod members (presumably including L-112) on the coast near the time of the stranding. Acoustic recordings of L pod calls indicated the presence of L pod near Point Reyes, CA on January 30, 2012, Fort Bragg, CA on January 31, 2012, off Westport, WA on February 5, 2012, and near Newport, OR on February 20 to 21, 2012. The acoustic data suggest that L-112 had likely been in central Oregon to northern Oregon-southern Washington waters at the time of her death. Members of L pod were photographed in Discovery Bay, WA on February 7, 2012 but L-112 was not seen there.

Weather and sea surface data for coastal Oregon and Washington waters (Feb. 1 to 11, 2012 coincident with the anticipated time of death of L-112) and drift patterns established for the Columbia River plume suggested the likelihood that L-112 had been carried for some days in the Columbia River eddies or drifted north from further south on the Oregon coast on the prevailing south winds and currents before being cast on the Long Beach Peninsula. These data support the hypothesis that L-112 was somewhere between central to northern Oregon when she died. No other killer whales were reported stranded in association with this case and there was no evidence of other stranded birds, mammals, or fish in the area at the time of L-112's discovery.

Based on findings from the gross examination of the carcass and absence of conclusive histopathology or ancillary test results, the investigative team found that blunt force trauma was the primary consideration for the death of the animal, but the nature of the blunt trauma could not be determined (i.e., L112 was hit, struck, or rammed in the head or neck but the animate or inanimate source of the blow could not be identified). NOAA received reports of sonar activities from hydrophone operators in Puget Sound preceding the stranding. Reporting parties expressed concerns that military activities may have been involved in the stranding. NOAA made inquiries for information on sonar, underwater explosive activities, and boat strikes in the Northwest to the Royal Canadian Navy, the United States Navy, the United States Army, and the United States Coast Guard. NOAA also requested information on human activities from additional organizations that operate or authorize activities off the coast. The Network investigators examined the circumstances of the stranding (single individual), environmental evidence, and information about human activities, and ruled out several possible sources of the traumatic injury, including the following:

1) Sonar and small underwater explosive activity was confirmed by the Royal Canadian Navy on February 4, 5, and 6, 2012 in Canadian waters off Vancouver Island and in the Strait of Juan de Fuca, but no marine mammals were observed during the training activities. The activities occurred too far to the north and downwind of the stranding location (prevailing winds and currents were from the south) to be a consideration.

2) The U.S. Navy reported that no naval training activities involving sonar or explosives were conducted between February 1 to 11, 2012 in the Northwest Training Range Complex (which includes Washington, Oregon, and northern California).

3) The U.S. Army reported no army-related training or military activities were carried out on the coast in early February.

4) Northwest Fisheries Science Center (NWFSC) hydrophone recorders off Newport, Oregon, and Westport and Cape Flattery, Washington did not detect sounds from mid-frequency sonar or explosions in early February.

5) The U.S. Coast Guard reported that no vessel-related whale strikes were recorded in early February from Oregon through Grays Harbor, Washington and they were not aware of any explosives being used in the area. No ship strikes of any whales in Oregon or Washington waters were reported to NOAA.

6) The Fishing Vessels Operators Association reported that no incidental mortalities or injuries involving killer whales were reported by commercial or recreational fishing vessels during January to February 2012. No reports involving killer whales were submitted to NOAA's Marine Mammal Authorization Program during this time period.

7) The Army Corps of Engineers reported that there were no in-water construction projects involving pile driving or explosive activities, nor were there any scientific buoy installations or dredging projects being conducted along the Oregon and Washington coasts in this time period.

In conclusion, blunt trauma to the head and neck is the prime consideration for the cause of mortality. Despite extensive diagnostic evaluation, the cause of the head and neck injuries could not be determined.

INTRODUCTION

The Northern Oregon/Southern Washington Marine Mammal Stranding Network received the report of a stranded female juvenile killer whale at 0700 on February 11, 2012, 0.9 miles north of Cranberry Road, Long Beach WA (Lat. = 46.40939 N; Long. = -124.06134 W). Keith Chandler of Seaside Aquarium, Seaside, Oregon responded, collected Level A data, including photographs (Figures 1 and 2), and had the animal moved (Figure 3) to a secure area in Cape Disappointment State Park to protect the carcass from vandalism and to provide a suitable site for necropsy. The whale was subsequently identified as Southern Resident killer whale L-112 based on photographs of the dorsal fin and saddle patch that biologists from the National Marine Fisheries Service (Seattle, WA) and the Center for Whale Research (Friday Harbor, WA) matched to catalogs of known killer whales. To fully investigate the stranding of L-112, a member of the endangered Southern Resident killer whale population, a multi-disciplinary team conducted a gross examination and full necropsy, including a suite of diagnostic tests. In addition, the team evaluated information on the sighting history of L-112 and environmental factors prior to the time of the stranding to identify the geographic area and timing of mortality. This report compiles all available information on L-112, the gross and histopathologic findings, and test results to inform our assessment of pathologic factors contributing to the whale's death. We also examine the context of the stranding, including both environmental factors and human activities, to further assess the potential cause of death.

BACKGROUND

The majority of Southern Resident killer whale (SRKW) sightings are documented during the spring and summer months (May through October) in the Salish Sea (marine waters of Washington and southern British Columbia east of the entrance to the Strait of Juan de Fuca). SRKWs increase their use of the outer coastal waters in the fall as evidenced by their lack of occurrence in the Salish Sea. SRKWs are observed intermittently from October to early January in Puget Sound. However, their last occurrence of the season in inland waters, particularly for L pod, can be as early as November. In addition, it should be noted that it is common for L pod to split up and travel for extended periods in subgroups.

Killer whale sighting information is gathered from a number of sources, including the Center for Whale Research, whale watchers, researchers, and citizens who report through sighting networks like Orca Network. The data is compiled into an “Orca Master” data set by the Whale Museum at Friday Harbor. The Center for Whale Research analyzes demographic and sighting data to compile an annual census of the SRKW population. Individuals in the population are identified using an alphanumeric code indicating kinship and birth order. Individuals may also be named.

L-112 (Sooke) was first photographed with her mother L-86 on February 6, 2009 off Victoria, British Columbia by K. Balcomb III. He estimated at that time that she was 6 to 8 weeks old and born in December 2008. She was possibly sighted earlier off Depoe Bay, Oregon on January 29, 2009, but there are no photographic records to confirm this sighting. L-112’s immediate family consists of L-86 (mother, age 21) and a 7 year-old male sibling, L-106. The extended family, which includes L-55, L-27, L-82, L-116, L-103, and L-109, forms a subgroup called the “L-4s,” one of several different subgroups of the larger L Pod.

The 2011 census of the Southern Resident killer whale population indicated that L pod numbered 42 individuals on December 31, 2011, prior to the stranding. Most of the time, members of L Pod do not travel together as a single group. During her lifetime, L-112 and her immediate family traveled with several family groups that represented roughly half of the population of L Pod. Consequently, the L-112 sighting information below includes her family but not necessarily all of L Pod.

After the initial sightings in January and February 2009, L-112 was not seen again until June 21, 2009. Sightings continued throughout the summer of 2009 and the last sighting occurred on October 9, 2009 off the west side of San Juan Island. There were no documented sightings of L Pod during the winter of 2009 to 2010. On May 25, 2010, L-112 was seen in Haro Strait during L Pod’s first summer appearance in the Salish Sea. The last sighting of L-112 in 2010 was December 6 off the south end of San Juan Island as the whales were heading west into the Strait of Juan de Fuca. During the winter, all members of L Pod were sighted several times between February 10 to 13, 2011 in the San Francisco and Monterey Bay areas. L-112 was next documented on May 29, 2011 in the Canadian Gulf Islands and photographed numerous times in Washington inland waters during the summer of 2011. The last images of L-112 prior to her stranding were taken on October 21, 2011 south of Discovery Island, British Columbia, heading for San Juan Island. Subsequent movements of L pod in 2011/2012, presumably including L-112, were detected by passive acoustic recorders and are shown in the Results: Relevant Factors subsection.

METHODS AND INVESTIGATORS

A multi-disciplinary team responded to the stranding, conducted a gross examination, and reported basic information on a Level A data sheet (Appendix A). Investigators conducted a necropsy, following a specific protocol developed by Raverty and Gaydos (2004), and collected a suite of samples for a variety of analyses to be performed at different reference laboratories. In addition, the investigators conducted computed tomography (CT) scans of the head, bones of the middle and inner ears, and post-skeletal preparation cervical spine, with a subsequent detailed head examination. Following the necropsy, the skeleton was cleaned and prepared for display. To put the examination and sample analysis results in an ecological context, the investigators collected and requested information on environmental factors and human activities. Below is a list of methods used to investigate the stranding of L-112 and information on the organizations and individuals that participated in different aspects of the investigation.

- Gross Exam and Necropsy:
    - Dr. Debbie Duffield, Portland State University, Lead Investigator
    - Cascadia Research Collective
    - Washington Dept. of Fish & Wildlife, Marine Mammal Investigations
    - Seaside Aquarium
    - Seattle Seal Sitters
    - Makah Fisheries Department
    - NOAA Fisheries
- Histopathology:
    - Oregon State University, Veterinary Diagnostic Laboratory
    - Animal Health Center, Abbotsford, BC, Canada
- Bacteriology:
    - University of California, Davis
    - Animal Health Center, Abbotsford, BC, Canada
- Virology:
    - University of California, Davis
- Toxicology:
    - Animal Health Center, Abbotsford, BC, Canada
- Contaminants:
    - NOAA Northwest Fisheries Science Center
    - Columbia Analytical Services (through Cascadia Research)
- Detailed Head Examination:
    - Dr. Joe Gaydos, University of California at Davis, Lead
    - Washington Dept. of Fish & Wildlife, Marine Mammal Investigations
    - The Whale Museum
- Computed Tomography:
    - VCA Veterinary Specialty Center of Seattle
    - Animal Internal Medicine and Specialty Services
- Stomach Content Analysis:
    - Biology Department, Portland State University
    - NOAA Northwest Fisheries Science Center

- Parasite Identification:
  - University of Florida
  - NOAA Northwest Fisheries Science Center
- Gas Analysis for Effects of Blast Trauma:
  - Woods Hole Oceanographic Institute
- Skeletal Preparation:
  - The Whale Museum
  - University of Washington, Burke Museum
- Relevant Environmental Factors and Human Activities:
  - NOAA Fisheries, Protected Resources Division
    - NOAA, Office of Response and Restoration, Emergency Response Division
    - U.S. Navy
    - Royal Canadian Navy
    - U.S. Air Force
    - U.S. Army
    - U.S. Army Corps of Engineers
    - U.S. Coast Guard
    - Fishing Vessel Owners Association
    - NOAA Marine Mammal Authorization Program
  - NOAA Northwest Fisheries Science Center
    - Center for Whale Research
    - Orca Network
    - The Whale Museum
- Law Enforcement Investigation:
  - NOAA Office for Law Enforcement

Requests for information on relevant factors included:

- L-112 life history and movements
- L Pod movements in 2011/2012
- L Pod demographics and population census
- Ocean currents and temperature, weather, and wind patterns
- Columbia River eddies and drift patterns
- Chinook salmon run timing
- Human activities, military exercises, marine mammal incidental take during fishing, vessel collisions, and in-water construction

RESULTS

Gross Examination and Findings

History: A post-mortem examination was performed on February 12, 2012, led by Northern Oregon/Southern Washington Marine Mammal Stranding Network primary responder Dr. Debbie Duffield (Portland State University). A complete photographic series of the necropsy has been archived by Portland State University, Washington Department of Fish and Wildlife, and Cascadia Research Collective. An initial brief necropsy report was prepared by Portland State University to accompany submission of histopathology samples, a comprehensive necropsy report with morphometrics and complete sample allocation was prepared for the group by Cascadia Research Collective and Washington Department of Fish and Wildlife, and a Marine Mammal Mortality Investigation Gross Report dated March 19, 2012 was prepared by D. Lambourn (Washington Department of Fish and Wildlife), J. K. Gaydos (SeaDoc Society / University of California Davis Wildlife Health Center / San Juan County Marine Mammal Stranding Network), D. Duffield (Portland State University), J. Huggins (Cascadia Research Collective), and T. McKlveen (VCA Veterinary Specialty Center of Seattle). These reports are included in Appendix A. The Southern Resident Killer Whale L-112 Stranding Progress Reports issued by NOAA on April 2, 2012 and May 12, 2012 are included in Appendix B.

External examination of L-112 revealed that the carcass was moderately distended with gas, and that there was minimal scavenging on the left, exposed side of the body (superficial, mostly from birds). The carcass was in moderate to late-moderate post-mortem condition (code 2.5 to 3.0). There are varying opinions on the exact state of decomposition because the abdominal cavity appeared more decomposed than the thoracic cavity and the skin was just starting to dry, peal, and slough. Based on the external examination, the initial estimated time of death was from 2 to 4 days to 1 week prior to discovery. The estimated window for time of death was later expanded to as long as 10 days based on the degree of post-mortem autolysis noted on histopathology.

External Examination: The whale was in good body condition with a 4.5 cm dorsal blubber thickness and fat noted around the heart. Throughout the ventrolateral aspect of the head, neck, and chest there was variably extensive red mottling of the skin. Along the left dorsolateral aspect of the head, subcutaneous bruising extended from approximately 5 cm above the eye rostrally to the level of tooth # 6 on the lower jaw, and caudally to the midlevel of the neck (Figure 5). Other smaller areas of hemorrhage were observed in the middle of the left eye patch and immediately anterior to the insertion of the left pectoral fin. Extensive bruising and swelling were also observed on the right lateral side of the head and neck (Figure 6) approximately 5 cm above the eye to the level of tooth #4 on the right lower jaw, and extended down the right side of the body, past the insertion of the right pectoral fin to the midlevel of the thoracic wall. Severe bruising spanned the ventral lower jaw, almost to the inside of the left lower mandible (Figure 6). The eyes were intact and slightly protuberant. Throughout the head, chest, and down the right lateral side of the body, there was extensive hemorrhage and edema in the skin, blubber, subcutaneous tissues, and muscles (Figure 8), as well as in the lung and heart. Hemorrhage was also noted in the thyroid gland; subscapular, suprascapular, mediastinal, and reproductive lymph nodes; tongue, esophagus oropharynx, and pharyngeal musculature; liver; and around the spinal cord at the foramen magnum and lumbosacral junction.

Two small, healed superficial longitudinal linear scars were present in the skin along the right torso: one immediately caudal to the trailing edge of the dorsal fin and the second was dorsolateral to the anus. Subcutaneous swelling was present around the genital slit (Figure 7) and the anterior margin of the blowhole was raised and swollen. The tongue was markedly swollen, congested, and edematous (Figure 5B), and a portion of the right side appeared collapsed. There were 12 teeth erupted from the right and left mandibles as well as from the right and left maxillae. On the left mandible, the tenth tooth was deviated more medially than the other teeth, but it appeared to have erupted in this direction because the tooth was firmly held by the periosteal ligament and there was no associated bruising, inflammation, or signs of trauma. The upper right tooth #13 had a vertical linear fracture which extended to the level of the gingival and had minimal separation of the opposed tooth margins.

Musculoskeletal System: No broken bones were observed on gross examination or upon flensing and cleaning the skeleton; however, anomalies were observed in the cervical vertebrae by CT imaging and bone preparations. Small splits were noted on some of the ribs after the skeleton was rearticulated. The splits run parallel to the length of the ribs from the vertebra to the sternum. Ante-mortem trauma of ribs would more likely produce transverse fractures (i.e. perpendicular, or at an oblique angle to the long axis of the bone. The observed splits are not consistent with ante-mortem fractures and are likely an artifact of skeletal preparation.

Digestive System: In the base of the mouth, medial to the right mandible and ventral to the tongue, there was a large, triangular 7-cm diameter dark green brown emphysematous area that protruded up to 16 cm above the surrounding tissues. A similar appearing and smaller area (~3 cm long) medial to the left mandible was also noted. Brown-green linear tracks extended from the mandibular symphysis, caudally towards the mandibular ramus and pharyngeal area. The mandibular or acoustic fat of the left mandible was dark red and the right mandible fat appeared more autolyzed and darker, suggesting passive congestion. Removal of the mandibles and the hyoid apparatus revealed air-filled sponge-like brown material immediately rostral to the tympanic bulla on the right, and to a much lesser extent on the left, side of the head. Frozen serosanguinous fluid (presumptive blood) was evident in the lumen of the oropharynx, as well as at the junction of the nasopharynx. The left pharyngeal muscles were hemorrhagic and edematous. The forestomach lining was diffusely detached, sloughed in one piece, and was compressed into a ball inside the lumen of the main compartment. In the glandular compartment, there was focally extensive erosion of the mucosa with a few 8 cm x 6 cm ulcers. Approximately 30 nematodes were interspersed within the detached forestomach lining and there was a moderate amount of ingesta within the small and large intestines. Feces were present in the colon.

Respiratory, Cardiovascular, Hemolymphatic, and Urogenital Systems:
Approximately 3 liters of dark red serous fluid was in the right, and to a much lesser extent in the left, thoracic cavities. Both lungs were congested, and with the right craniobronchial lobe there was moderate focally extensive subpleural edema and hemorrhage. There was approximately 50 ml of dark red-black serous fluid within the pericardium, and the epicardium was edematous (Figure 9). On the cut surface the myocardium was green (autolysis) and there was no blood within the lumen of the heart or peripheral large caliber blood vessels (Figure 9), suggestive of hypovolemia, secondary to hemorrhage in the head and neck regions. Although tissue gas accumulation and compression of the heart and great vessels may have displaced the blood cranially into the neck and tongue (bloat), it is also possible that hypostasis because of position in the water column prior to stranding may also account for the lack of blood. The kidney and spleen were friable and tan brown (autolysis). The urinary bladder was empty.

Nervous System: At the time of necropsy, the atlanto-occipital junction was transected, and the head was frozen intact. Imaging studies were conducted February 23, 2012 and the head was dissected March 6 to 7. On initial incision of the neck, large amounts of dark red serous fluid (~2 L) and variably sized fragments of brain poured from the foramen magnum (Figure 10). The head dissection (Appendix A) revealed extensive subcutaneous bruising around the left, and to a lesser extent right, eyes. The fascia and blubber surrounding the melon (dorsally and laterally) was diffusely pink to red. There was more intense red discoloration of tissues immediately rostral to the blowhole and lateral diverticulae (multiple sacs associated with the blowhole), which extended up to 27 cm towards the snout. The pink to red color was darker on the right side than it was on the left. The right craniofascial muscles adjacent to the melon, immediately dorsal to the maxilla were dark red. Focally extensive hemorrhage was evident in the connective tissue on the right side of the head at the junction of the blowhole's rostral vestibular sac and the melon (Figs 2 and 6). Approximately 5 to 10 cc of serosanguinous fluid was present frozen in the left nares within the blowhole, likely an artifact of the head's position when frozen, and the right nares was clear.

Dissection of the tympanic bullae revealed that the right bulla was less firmly attached to the skull and significantly looser than the left bulla. After removal of the tympanic bullae, 1 small (1 to 2 cm) nematode and approximately 12 slightly longer (2 to 4 cm) worms were found in the area of the skull adjacent to the tympanic bulla, including peribullar sinus, fibro-venous plexus, and surrounding peribullar soft tissue of both bullae. Red serous fluid was present in both peribullar sinuses. Dorsal to the right and left bullae, there were two small bony fragments. The left fragments were approximately 2.5 cm x 2 cm and 1.5 cm x 1.5 cm and the right fragments measured 4 cm x 2 cm and 1.5 cm x 1.5 cm and were displaced into the calvaria. The edges of all four pieces were irregular but well-rounded and did not appear to be fractured bone fragments or remnants. Removal of a large triangular section of the occipital bone for access to the calvaria revealed cerebrum encapsulated by meninges in the left side and a large portion of dura that was adherent to the calvaria in three to four places. The right cerebellum and cerebrum were mostly lost, with portions of brain tissue draining into the left bulla. Roughly 20 cc of dark red to brown serosanguinous fluid was between the dura and the calvaria (epidural). This fluid was consistent with the fluid that was noted during the initial necropsy. The sutures on the right side of the calvaria appeared to be looser than on the left side and red serous fluid was leaking through the suture areas.

Histopathology

Histopathology was performed on tissues harvested at the time of necropsy and subsequent to the head dissection. Severe autolysis hampered microscopic assessment of virtually all examined samples. In the multiple lymph nodes and throughout the small intestine and mediastinum, there were numerous microcavitations (emphysema) with no associated bleeding (hemorrhage) or inflammatory infiltrate. As fat emboli were also a consideration for the clear areas, cryostat sections were prepared of affected tissues and stained with Oil Red O. There was no indication of fat embolization in any of the examined tissues. Varying amounts of bacterial overgrowth were observed throughout the tissues. Within the heart muscle, kidney, and liver, there was a small amount of scar tissue and in a few kidney tubules there were scattered protein casts (tubuloproteinosis). Histologic sections of the tissues associated with the right bulla disclosed moderate inflammatory infiltrate. Sections of the nonglandular stomach compartment revealed pronounced thickening of the inner lining (stratified squamous epithelia), and in few areas there were intranuclear inclusions suggestive of an underlying viral infection. Throughout the liver there was moderate biliary ductular hyperplasia with occasional bridging, periductular fibrosis, and cholestasis. A small number of skeletal muscle fibers featured intracellular protozoa morphologically consistent with *Sarcocystis* spp. and in a few sections of skeletal muscle there was variably extensive interstitial accumulation of acellular to hypocellular proteinaceous material. No other significant findings were identified within the examined tissues. Histopathology reports are included in Appendix C.

Computed Tomography of the Head and Bullae

On February 23, 2012 CT scans were performed on the intact head and subsequently on the separated bullae (Appendix E). Findings from the scans of the entire head indicated extensive gas accumulation in the soft tissues and fat. The absence of right cerebral hemisphere and right cerebellum of the brain was secondary to loss of tissue during disarticulation of the head. Significance is uncertain based on imaging alone, but unilateral loss of brain tissue is unusual. Bilaterally, small mineralized deposits were detected dorsal to the bulla and suggestive of otoliths, dystrophic mineralization, or parasitic granulomas with right-sided displacement through the foramina into the calvaria possibly because of loss of supporting tissues. In the left, and to a much lesser extent right, osseous bullae (middle ears), there was fluid or soft tissue accumulations, which may be attributed to blood, infectious pathogens, nematodes, inflammatory debris, polyp-like material, post-mortem accumulation of fluid, or engorged mucous membranes.

Sinusitis was noted and likely related to parasite migration.

Microbiology, Molecular Studies (polymerase chain reaction), Trace Mineral, Contaminants, and Biotoxin Analyses

Bacterial overgrowth secondary to post-mortem decomposition likely hindered recovery or detection of any significant pathogens. Lung, spleen, lymph node, brain, cerebrospinal fluid, meninges, liver, colon, blowhole and mammary gland swabs, and kidney were submitted to two reference laboratories for routine and special microbiology (Appendix D). Small to moderate growth of *Edwardsiella tarda* were recovered from select tissues, a few *Micrococcus* sp. were recovered from the spleen, and moderate growth of alpha *Streptococcus* sp. were cultured from the colon. No *Salmonella* sp., *Campylobacter* sp., or *Yersinia* sp. were recovered in selective media. Anaerobic culture recovered large numbers of *Clostridium perfringens*, *C. sordelli*, and *C. difficile* from the colon, and heavy growth of *C. septicum* was isolated from the discolored skin. Because of the grossly noted hemorrhage and emphysema within the neck and head skeletal musculature, immunoflorescence for *Clostridial* toxins to rule out clostridial myositis was pursued and proved negative for *C. chauvoei, C. noyvi,* and *C. sordelli*. Polymerase chain reaction (PCR) of pooled tissues proved negative for herpesvirus, *Brucella spp.*, canine distemper virus, West Nile virus, and influenza virus, and results from trace mineral and vitamin A analyses of the liver were within acceptable reference limits.

Liver and kidney tissues were analyzed by Columbia Analytical Services in Kelso, Washington (contracted by Cascadia Research) and Prairie Diagnostic Laboratories, Saskatoon, Saskatchewan for the following elements: aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), methyl mercury (MeHg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), and zinc (Zn). Results are presented in Table 1. Tests on the ingesta for biotoxins, domoic acid, and saxitoxin were negative and a test stripe for *Cryptococcus* sp. did not detect antibodies in post-mortem heart blood.

PCR of the heart proved positive for Apicomplexa (NIH, Dr. M. Grigg); however, close evaluation of the myocardium did not reveal any discernible protozoa.

The Northwest Fisheries Science Center completed an analysis of persistent organic pollutants (POPs; e.g., DDTs, PCBs (and congeners), chlordanes, and polybrominated diphenyl ethers (PBDEs)) (Appendix D). The relative percentages of five lipid classes in the blubber and POP concentrations are shown in Tables 2 and 3. The sample had no free fatty acids present, which indicates that the blubber sample was not subject to decomposition significant enough to alter toxicological analysis (NWFSC 2013). The blubber/skin decomposition does not always correlate with decomposition noted for internal organs. Internal organs of the animal are more likely to decompose because of increased temperature in the carcass compared to the outer most tissues (e.g., blubber).

Table 1. Trace metal concentrations in liver and kidney tissues of L-112. Results are parts per million (ppm), dry weight.

		L112			**Odontocetes**		**Mysticetes**	
		CAS*		PDS	Range**		Range**	
Element	Unit	Liver	Kidney	Liver	Liver	Kidney	Liver	Kidney
Aluminum	dw, ppm	3.1	10.2					
	ww, ppm	0.93	3.06				6.4-150	1.6-9.3
Arsenic	dw, ppm	0.56	0.83					
	ww, ppm	0.168	0.249				0.47-0.7	0.01-2.7
Cadmium	dw, ppm	0.812	7.98		<0.05-175	<0.05-426	0.56-18.61	1.93-208.9
	ww, ppm	0.244	2.39		nd-11.5	2.19-18.0	0.06-6.2	0.14-6.1
Calcium	ww, ppm			2				
Cobalt	ww, ppm			0.009				
Copper	dw, ppm	28.2	7.33		5.3-260	5.7-73	3.37-228	7.68-65.0
	ww, ppm	8.46	2.2	9	6.02-16.3	1.68-4.53	0.63-25	0.45-4.9
Iron	ww, ppm			175				
Lead	dw, ppm	0.0755	0.0387		<1.0-3.2	<1.0-3.6	0.78-3.62	0.34-6.12
	ww, ppm	0.0227	0.0116				0.02-0.27	nd-0.1
Magnesium	ww, ppm			213				
Manganese	ww, ppm			2				
Mercury	dw, ppm	33.8	7.94		0.6-344	0.9-105		
	ww, ppm	10.1	2.38	12.4	0.31-97.8	0.21-10.4	0.009-0.12	nd-0.06
Methyl mercury	dw, ppm	1.83	1.98		0.8-3.0	0.2-2.0		
	ww, ppm	0.549	0.594					
Molybdenum	ww, ppm			0.28				
Nickel	dw, ppm	0.38	0.38		0.2-1.5	<0.2-1.2	1.53-2.13	1.07-3.29
	ww, ppm	0.114	0.114				nd-0.35	nd-0.21
Selenium	dw, ppm	21.4	8		0.6-99	0.1-57		
	ww, ppm	6.42	2.4	5.18			0.35-3.4	0.34-2.1
Silver	dw, ppm	2.73	0.061					
	ww, ppm	0.819	0.0183				0.01-0.02	0.01-0.03
Zinc	dw, ppm	306	121		40-684	44-201	59.07-209.09	66.21-212.15
	ww, ppm	91.8	36.3	98	59.8-93.5	15.3-30.9	1.6-160	32-110
Vitamin A	ug/g			1844.4				
Vitamin E	ug/dl			829.2				

*Results from CAS were dry weight (dw), and were converted to wet weight for comparison to literature using average moisture content of 70%.

**Values from literature were not converted; wet and dry weight range values for a single element are from different sources.

Table 2. Animal information, total lipid, and lipid classes in blubber (0-2 cm depth) of L-112 and some other juvenile Southern Resident killer whales.

Pod ID number	Age at sampling	Age class	Sex	Collection date	% Lipid TLC-FID†	% of total lipid SALE	TG	FFA	CHOL	PL
L112*[a]	3 yrs	Juvenile	Female	2/12/12	51.73	19.8	80.2	0.0	0.0	0.0
L98*	4 yrs	Juvenile	Male	3/13/06	56.39	13.1	83.2	2.7	0.0	0.0
J39¥	3 yrs	Juvenile	Male	5/23/06	40.85	13.8	84.4	1.8	0.0	0.0
J38¶	4 yrs	Juvenile	Male	6/8/07	20.90	16.1	81.1	2.7	0.0	0.0
K36¶	4 yrs	Juvenile	Female	12/14/07	18.30	14.1	81.5	4.4	0.0	0.0
K42[a]	4 yrs	Juvenile	Male	11/27/12	27.60	16.9	83.1	0.0	0.0	0.0

† Percent lipid and lipid classes determined using thin-layer chromatography/flame ionization method (Ylitalo et al., 2005)

SALE = stearic acid laurel esters; TG = triglycerides; FFA = free fatty acids; CHOL = cholesterol; PL = phospholipids

* Necropsy blubber sample; other samples biopsy blubber samples

¥ Data from Krahn et al., 2007

¶ Data from Krahn et al., 2009

[a] Unreported data from NWFSC, 2014

Table 3. Concentrations of persistent organic pollutants in blubber (0-2 cm depth) of L112 and some other juvenile Southern Resident killer whales.

Pod	**ng/g, wet weight**						**ng/g, lipid weight**					
ID number	**HCB**	**∑CHLDs**	**∑DDTs**	**∑HCHs**	**∑PCBs**	**∑PBDEs**	**HCB**	**∑CHLDs**	**∑DDTs**	**∑HCHs**	**∑PCBs**	**∑PBDEs**
L112*[a]	630	4,100	31,000	390	20,000	2,400	1,200	7,900	60,000	750	39,000	4,600
L98*	250	4,600	44,000	300	24,000	1,900	440	8,200	78,000	530	43,000	3,400
J39¥	650	2,100	9,800	530	14,000	6,000	1,600	5,100	24,000	1,300	34,000	15,000
J38¶	250	1,100	5,000	210	8,600	3,000	1,200	5,300	24,000	1,000	41,000	14,000
K36¶	360	2,200	17,000	320	11,000	2,800	2,000	12,000	93,000	1,700	60,000	15,000
K42[a]	180	770	5,400	100	4,700	970	650	2,800	20,000	360	17,000	3,500

* Necropsy blubber sample; other samples biopsy blubber samples

¥ Data from Krahn et al., 2007

¶ Data from Krahn et al., 2009

[a] Unreported data from NWFSC, 2014

Gas Examination

Because of concerns of possible blast injury and seismic or sonar exposure, coupled with the grossly noted gas accumulation in multiple tissues on necropsy, four samples of air bubbles from the heart and two aliquots from the mesenteric vein were collected in BD vacutainers and a monoject tube (Bernaldo de Quiros et al. 2011) and, shipped in a sealed, pressurized container with a barometer and altimeter to Dr. Yara Bernaldo de Quiros Miranda, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts for Mass Spectrometry analysis. Gas bubble analysis yielded consistently higher carbon dioxide to hydrogen and nitrogen levels in all samples, compatible with putrefactive gases. However, the animal was considered too decomposed to detect gas embolism chemically (Bajanowski et al. 1998; Bernaldo de Quiros et al. 2011; Pierucci and Gherson 1968; Pierucci and Gherson 1969) (Appendix D).

Parasitology

The nematodes found in the right bulla were identified as *Crassicauda* sp. by Dr Heather Stockdale Walden of the University of Florida, College of Veterinary Medicine (Appendix D), and the helminths recovered from the forestomach were *Anisakis* sp. *cf. A. simplex.*

Food Habit Information

Ingesta from stomach and intestines, and feces from the colon of L-112 were collected at necropsy and examined for prey remains. The forestomach contained 17 cc of brown mucoid fluid. The main and pyloric chambers contained approximately 35 cc of pink salmon-colored pasty material with six small fish eye lenses. A fecal sample obtained from the colon was screened genetically for all prey species known to be in the diet of resident-type killer whales (see Hanson et al. 2010 for methods). Only Chinook salmon and halibut DNA were detected. Stock-specific information for the salmon could not be determined as the Chinook salmon DNA was too degraded (Hanson et al. 2010).

Skeletal Examination

Skeletal flensing, cleaning, and disarticulation of the skeleton revealed no bone fractures. However, CT scan of the cervical vertebrae detected a defect in the dorsal lamina and no dorsal spinal process of C7. Based on follow up gross examination of the vertebra, it was concluded that this malformation was likely congenital and pre-existing the stranding and likely not because of physical trauma. In the right maxilla, there was a vertical crack of tooth #13 which extended from the apical tip to below the dental ligaments (Figure13). There was no indication of odontodystrophies, maxillary osteomyelitis, developmental anomalies, periodontal disease, caries, or host response to the defect. The cause of the crack is undetermined. Shearing chips, consistent with biting, were also observed on some teeth. Tooth #6 on the right mandible showed the most dramatic chip, but the cause was not determined.

Relevant Historical Factors

L Pod movements in 2011/2012: In fall 2011, the NWFSC deployed seven autonomous passive acoustic recorder moorings off the coasts of Washington, Oregon, and California (Figure 4) and these arrays were recovered in the summer and early fall of 2012. The recorders were set to record for 30 seconds every 10 minutes during their deployment. The recorder moored off of the Columbia River failed in mid-November 2011. The mooring for the Cape Flattery inshore recorder failed in early February and the recorder was recovered near Tofino, British Columbia in April 2012.

In 2011, L pod was last sighted in inland waters with J and K pods on November 2, off Race Rocks, Canada. On December 15, 2011, S19 calls (used by all members of L pod) were detected on the recorder moored near the head of the Juan de Fuca Canyon about 10 miles west of Cape Flattery, Washington. The next detection of L pod was on January 26, 2012 on the recorder moored a few miles southwest of Fort Bragg, California. On January 27, L pod-specific calls were detected on another NWFSC recorder located a few miles southwest of Point Reyes, California. Additional L pod calls were again detected on the Point Reyes recorder on January 30 and then on the Fort Bragg recorder on January 31. This series of detections (which, unlike 2011, were short in duration) indicates relatively quick southbound movement of the whales on January 26 to 27th and subsequent northbound movement on January 30 to 31. Call types heard indicate the main group of L pod animals was present, which could have included L-112. Between 08:00 and 09:00 on February 5, 2012, S16 calls (used by both K and L pods) were heard on the recorder located about 5 miles west of Westport, a distance of 531 miles north of the Fort Bragg recorder, indicating an average speed of approximately 4.5 miles per hour if this was the same group of whales. After the December 15, 2011 detection the Cape Flattery offshore hydrophone did not detect SRKW calls again until March 4, 2012.

Also on February 5, hydrophones on the west side of San Juan Island detected K and L pod calls near Limekiln Point at 10:55 and Andrews Bay later that evening (22:45). The traveling distance between Westport and Limekiln Point (200+ miles) is too far for the group heard at Westport to travel and be detected at Limekiln that same morning, indicating that at least two groups of K and/or L whales were transiting in Washington waters on February 5.

Part of K pod (K18s and K16s) and part of L pod (L2s, L9s, L54s) were photographed during an unprecedented appearance in Discovery Bay, Washington on February 7, 2012 at 15:45. The L4s (the sub-group including L-112's matriline) were not seen with this group. It is not known whether the group observed in Discovery Bay included individuals from the group recorded off Westport on February 5 or the group detected at San Juan Island that same day. If the animals seen in Discovery Bay came from the outer coast group, the travel speed to cover the 233 mile distance between Westport and Discovery Bay during the intervening time would have averaged 4.3 miles/hour. This is within the range of the average traveling speed for SRKWs and similar to the 4.5 miles per hour rate of travel calculated for the trip between Fort Bragg (January 30 to 31) and Westport (February 5) or that is typically seen in their summer/fall range.

On February 20 to 21, 2012 SRKWs (K and/or L pods) were detected on the Newport, Oregon recorder. The next detection of L pod was on February 27 on the Westport, Washington recorder which was the beginning of an approximately 2-month period in which there are 16 SRKW detections and one visual sighting off Westport and one additional acoustic detection off Newport in mid-March. The acoustic recordings support the hypothesis that a group of whales possibly including the L4 sub-group and L-112 were present and could have been transiting in the area of the Columbia River plume during the time frame of the mortality and subsequent stranding.

Between the stranding date (February 11) and July 1, 2012, all of the remaining members of the L4 sub-group (L-27, L-55, L-82, L-86, L-103, L-106, L-109, and L-116) except L-112 were observed alive and were included in the 2012 census.

Wind and Currents: We reviewed weather and sea surface data for the coastal waters of Washington and Oregon from February 1 to 11, 2012 (the period from the estimated time of death until the stranding date) and found that prevailing winds and currents were predominantly from the south in the days prior to the stranding (Appendix F). Using current models and data from drift card studies, NOAA Office of Response and Restoration, Emergency Response Division (ERD) advised that current conditions off the Long Beach Peninsula, Washington are largely influenced by eddies created by flows from the mouth of the Columbia River. In the period prior to the stranding, eddies would have flowed northward under the influence of the prevailing currents, allowing floating debris trapped in them to be deposited on Long Beach.

ERD further advised that floating debris arriving from the open sea to the west or north of Long Beach would have been carried northward by the current to be deposited elsewhere on the Washington or British Columbia coasts, not on Long Beach near the mouth of the Columbia River. Figure 11 depicts patterns of surface drifters deployed by the University of Washington off the mouth of the Columbia River in 2005. These patterns illustrate the eddy circulation in the region. The cyan surface drifter tracks (from August 17) represent conditions that are most similar to the winds and currents off the Washington and Oregon coasts in February 2012. The tracks further substantiate the potential for objects floating in the plume to be deposited on Long Beach. Moreover, drift patterns from the prevailing winds and currents for this period indicated a northward flow along the Washington and Oregon coasts so that a floating object from far off of the Washington coast or farther to the north would be unlikely to have been deposited on the southern end of the Long Beach Peninsula.

Earthquakes: NOAA Fisheries received comments requesting that they investigate the possibility that earthquake(s) may have contributed to the stranding. Minor earthquakes (magnitude 2.6 to 4.0) were detected off the Oregon coast near Coos Bay on January 22, February 2, 3, 4 and 7, 2012 (earthquaketrack.com). A light magnitude 4.8 earthquake was recorded off Vancouver Island, Canada on February 4, 2013. Minor earthquakes are considered common in the region and some can be felt but seldom cause damage. There is little data currently available to assess the impact or possible contributions of small magnitude earthquakes to killer whale strandings.

<u>Sea Surface Temperature:</u> Sea surface temperature data between February 1 through 11, 2012 were obtained using NOAA's Comprehensive Large Array-Data Stewardship System (CLASS). Sea surface temperature is defined as the skin temperature of the ocean surface water and is generated every 48 hours for North America. Three locations were investigated: approximately 37 miles off southern Oregon, approximately 38 miles offshore of central Oregon, and the mouth of the Columbia River. The average sea surface temperature at each location for the period February 1 through February 11, 2011, 2012, and 2013 are presented in Table 4. Sea surface temperature data from each 48 hour cycle is available at:
http://www.nsof.class.noaa.gov/saa/products/welcome;jsessionid=932383178D182AB582750D19C760E4E4.

Average sea surface temperatures varied little (<1° C) and no large-scale temperature anomalies were noted between years 2011to 2013.

Table 4. Average sea surface temperatures (SST) offshore of southern, central, and northern Oregon between February 1 and February 11 for the three years 2011 to 2013.

Lat x Long	**Location**	**Average SST 2011**	**Average SST 2012**	**Average SST 2013**
43 x -125.2	southern Oregon	9.90°C (49.8°F)	9.86° C (49.7°F)	10.0°C (50.0°F)
45 x-124.8	central Oregon	9.76° C (49.5°F)	9.06° C (48.3°F)	9.73°C (49.5°F)
46 x -124.0	Columbia River	9.16°C (48.4°F)	8.96° C (48.1° F)	9.50°C (49.1°F)

<u>Acoustic Recordings and External Inquiries for Information:</u> On February 6, 2012, researchers monitoring hydrophones deployed in the inland waters of Washington detected sounds identified as military mid-frequency sonar and possibly explosions. The researchers linked the sounds to a Canadian Navy exercise in the Strait of Juan de Fuca involving the HMCS Ottawa. The researchers accessed Automatic Identification System data from Marine Traffic to retrace the movements of the HMCS Ottawa as it departed and returned from the North Pacific off Vancouver Island in the days prior to the exercise in the Straits. Reports of the sonar detections and accompanying impulsive sounds were published in the media and prompted considerable public interest and concern over potential sonar impacts to SRKWs. The concern intensified with the discovery of L-112 stranded on Long Beach 5 days later on February 11.

NOAA Fisheries solicited information from a variety of sources to identify whether human activities may have contributed to the injuries observed during the post-mortem examination and diagnostic assessment of L-112. Activities of particular interest included military exercises, vessel traffic and collisions, incidental take in fisheries, in-water construction activities involving demolition or blasting, and earthquakes, particularly in the 10 to 30 days preceding the stranding (Appendix F).

NOAA Fisheries requested information on naval activities from the Royal Canadian Navy. The Canadian Navy confirmed the use of sonar and small under water charges in Canadian waters west of Vancouver Island and in the Strait of Juan de Fuca. On February 4, Canadian naval exercises using a small (1.4 kg) explosive charge and sonar were conducted in Canadian waters approximately 85 miles northwest of the Strait of Juan de Fuca. According to the Navy report, the "kill radius for a human diver from the type of charge used is approximately 15 yards." Sonar was operated for approximately 8 hours at this general location. A similar exercise occurred approximately 90 miles northwest on February 5 when two small charges were deployed, one in the morning and one in the afternoon, and sonar was operated for approximately 11 hours in this general location. After the offshore exercises, the HMCS Ottawa returned to the Strait of Juan de Fuca using sonar while in transit to Constance Bank. On February 6, 2012, two small explosive charges were deployed in the morning as part of an anti-submarine warfare exercise near Constance Bank. In each case, the HMCS Ottawa adhered to their Marine Mammal Mitigation Policy prior to deploying the small charges and while using ships' sonar. Marine mammals were not detected in the area of the exercises by shipboard lookouts nor passive sensors, according to the Navy's report (Appendix F).

NOAA Fisheries also contacted the U.S. Navy regarding naval exercises in the Northwest Training Range Complex. The U.S. Department of the Navy, Office of the Chief of Naval Operations responded to our request and confirmed from their records that there were no naval exercises involving mid-frequency sonar use or explosives deployment in the Northwest Training Range Complex between the end of January and February11, 2012. The offshore area of the Northwest Training Range Complex includes surface and subsurface operating areas extending generally west from the coastline of Northern California (approximately Cape Mendocino), Oregon, and Washington for a distance of approximately 250 miles (463 km).

NOAA Fisheries contacted the U.S. Army to determine whether any military assets (Army, Air Force) from Joint Base Lewis-McChord (JBLM) may have been involved in training or other military activities on the coast during the time prior to the stranding. The Chief of Staff at JBLM responded that no military training, shipping, in-water construction, explosive events, or other potential perturbations involving JBLM units took place in the area during that time frame.

NOAA Fisheries also contacted the United States Coast Guard. The Coast Guard conducts search and rescue activities, homeland security and law enforcement tasks, maintains aids to navigation, and trains for these missions in the Lower Columbia River and marine waters off the coasts of southern Washington and northern Oregon. NOAA contacted them to see whether training exercises or civilian activities (e.g., shipping, resource exploration, fisheries, or in-water construction) along the Oregon coast as far north as Ledbetter Point, Washington may have been conducted by or reported to the Coast Guard between February 1and February 11. The Coast Guard reported that they did not learn of any whale strikes or other impacts to whales by Coast Guard assets or commercial vessels during this time frame. During that period, Coast Guard activities included search and rescue, law enforcement, aids to navigation work, and training exercises. Small boats and cutters made over 100 voyages in the area and on the Columbia River, Oregon. No major cutters transited the area and there were no reported whale strikes. There were 116 large vessel (over 300 gross tons) movements that arrived or departed Sector Columbia River's area of responsibility, which encompasses the Oregon coast north to Grays Harbor, Washington. Additionally, they are not aware of any explosives being used in the area during that period.

Regarding fisheries activities that may have encountered the members of L pod, NOAA Fisheries reviewed reports received by the Marine Mammal Authorization Program from commercial fishing vessels between January and February 2012 and found that no incidental mortality or injuries involving killer whale(s) was reported anywhere on the west coast during this time frame. We also contacted the Fishing Vessel Owners Association to see if they had received any reports of encounters with whales. The Fishing Vessel Owners Association responded that vessels are not typically on the water and fishing in February, and reported no interactions between whales and fishing vessels. Vessels that are part of the Association are smaller, between 50 to 85 feet in length, with crews of three to six persons, and travel an average speed of 7 to 12 knots.

The United States Army Corps of Engineers (USACE) is authorized by Congress to regulate activities that may impact wetlands and waters of the United States. In response to NOAA Fisheries' request, the U.S. Army Corps of Engineers' Regulatory Office based in Portland, Oregon provided information on permit actions along the coast of Oregon north to Leadbetter Point, Washington for the 5-year period prior to February 2012. Permit activities in the area were primarily beach grass removal on dunes, installation and maintenance of scientific buoys, and dredging. Permits for dredging activities are typically issued with special conditions and require additional consultation if marine mammals frequent the area in which the work will be completed. During the time frame of January 2012 through February 2012, the USACE was not contacted by any permit holder notifying them that marine mammals were present in the permitted work area. There were no permitted in-water construction projects involving blasting or pile driving in the coastal waters of Oregon or near the Columbia River mouth that would have produced noise disturbance during the time frame we requested.

Law Enforcement Investigation: An initial investigation into cause of death was undertaken by the NOAA Office of Law Enforcement. No subjects or witnesses with knowledge of the circumstances associated with or leading to the death of L-112 were identified. The case was closed due to a lack of evidence to support that a crime occurred.

DISCUSSION AND CONCLUSIONS

Killer whale strandings provide a rare opportunity to improve our understanding of natural history, diet, reproduction, threats, disease, and mortality. With the listing of the SRKWs as endangered in 2005, interest and resources have increased the number of complete investigations of killer whale strandings, particularly along the west coast of the U.S. (Barbieri et al. 2013). Stranding investigations were identified as an important action in the Southern Resident Killer Whale Recovery Plan (NMFS 2008). This report draws on a multi-disciplinary investigation to provide unique insight into the status of killer whales and evaluate potential threats and sources of mortality.

On February 11, 2012, a juvenile female Southern Resident killer whale, L-112, stranded just north of Long Beach, Washington. The whale appeared to be in good nutritional condition with advanced post-mortem decomposition. Gross examination revealed extensive subcutaneous bruising on the dorsolateral aspects of the head, tracking to the throat and anterior insertion of the right pectoral fin. Microscopic assessment of sampled tissues was hindered because of advanced post-mortem autolysis; at the time of the post-mortem examination, generalized gas accumulation in most major organs was noted, and based on this observation gas was collected and shipped to WHOI for analysis. Chemical analysis results indicate that the gas composition was most consistent with putrefaction (bacterial degradation) rather than gas bubble-like disease or blast injury. The lack of associated bleeding, inflammatory infiltrate, or fat embolization with the microcavitations in examined tissues further substantiated the interpretation of post-mortem decomposition.

During skeletal preparation, a congenital defect or anomaly of the dorsal aspect of cervical vertebra C7 (the lamina) was observed, with incomplete fusion of the lamina and incomplete formation of the spinous process (Figure 12). The cervical vertebrae were examined by CT scan and it was determined that this malformation was not due to a traumatic fracture (Appendix E).

Head imaging studies (CT scans) (Appendix E) and gross dissection showed disruption of the right cerebral hemispheres with marked accumulation of clear fluid, variably extensive hemorrhage, and collapse of the dura. Microscopic examination of brain sections disclosed tissue fragmentation and breakdown with no associated hemorrhage, fluid accumulation, or protein loss. These changes were consistent with freeze artifact and tissue breakdown because of post-mortem decomposition (autolysis) rather than a traumatic insult. Imaging studies also detected multiple bone fragments with soft tissue associated with the left ear bullae, and gas was noted in the right bullae. Conclusions from the CT scan of the right and left bullae at 1-mm slices did not show any evidence of fractures, dislocation, or crushing. The soft tissue or fluid attenuating material in the cochleae could be either pre- or post-mortem. There was no definitive evidence of acoustic damage to the boney ear structures of this whale identified from the CT study.

Histopathology of the right peri-bullar tissue disclosed chronic inflammatory infiltrate with associated *Crassicauda* spp. nematodes. Parasite infections of the inner ears have previously been documented in a number of cetaceans and the contributions of these worms to impaired sound perception or disequilibrium is unknown. In this case, the inflammation and parasitic burden would likely not have resulted in clinical disease.

Additional microscopic findings included low to intermediate grade accumulation of scar tissue within the heart, liver and kidney, which cumulatively would not have contributed significantly to antemortem morbidity. There was suggestion of an ascending infection from the gastrointestinal tract, possibly because of hepatobiliary trematodiasis or toxic exposure. Thickening of the stomach lining was also apparent (gastric hyperkeratosis) and suggested inappetance or anorexia. *Anisakis* sp. cf. *A. simplex*, was also identified in the stomach and was not considered pathologically significant. The fluid accumulation within the left chest cavity and lung was most likely due to autolysis, although bleeding and edema fluid related to trauma, impaired heart function, or active inflammation may also be considerations (Appendix D). Acute inflammation was noted in a small number of lymph nodes and was consistent with a low grade localized bacterial infection.

Aerobic culture of the sampled tissues yielded variable light to heavy growth of *Edwardsiella tarda* from the lung, lymph node, brain, liver, kidney and blowhole. This bacterium comprises part of the normal intestinal flora of killer whales with secondary invasion and septicemia typically diagnosed in animals with generalized debilitation or immunosuppression. Because of the extent of post-mortem decomposition and lack of associated inflammatory infiltrate, this isolate was likely due to post-mortem tissue invasion and proliferation. Selective culture of the colon did not recover *Salmonella* spp, *Campylobacter* spp, or *Yersinia* spp. Similarly, the large numbers of *Clostridium perfringens, C. sordelli*, and *C. difficile* recovered from the colon by enrichment broth likely represent post-mortem overgrowth. Because of the gross appearance of the subcutaneous bruising and gas accumulation, immunoflorescence was undertaken to screen for clostridial toxins (blackleg) and results proved negative for *C. septicum, S. sordellii, C. noyvi*, and *C. chauvoei*.

Molecular studies were undertaken to screen tissues for potential pathogen exposure, including *Brucella* spp., canine distemper virus (morbillivirus), influenza virus, West Nile Virus, and herpesvirus. Results were negative for these pathogens; however, because of the extent of post-mortem decomposition and DNA/RNA degradation, the possibility of false negatives cannot be entirely discounted. PCR of the heart proved positive for Apicomplexa (NIH, Dr. M. Grigg); however, close evaluation of the myocardium did not reveal any discernible protozoa. This parasite has previously been detected in stranded killer whales and is associated with massive die-offs of southern sea otters in California, and harbor seals and California sea lions in the Pacific Northwest. There was no indication of active protozoal infection or inflammation in L-112.

Metal concentrations in liver and kidney were compared to compiled ranges of published values for odontocetes and mysticetes (Varnassi et al. 1994; Sanpera et al. 1996; Holsbeek et al. 1999; Ruelas et al. 2000; Mendez et al. 2002; Das et al. 2004a and b; Endo et al. 2007). All elements were within the ranges of other cetaceans, and most compared to what has been recorded in other odontocetes. Nickel, however, was most comparable to mysticete values, which are higher than those of odontocetes. Some difference in derived levels between labs may be attributed to more labile or volatile trace minerals, which may be lost during transport and processing. In some laboratories, total mercury and total arsenic are analyzed, rather than inorganic and organic quantification. Acid extraction of the tissues in the processing phase may also artefactually increase select trace mineral concentrations. The anatomic sample location within an organ may also contribute to variations in heavy metal levels, such as copper levels throughout the liver, or lead partitioning in the renal cortex relative to medulla. None of the heavy metal findings are considered significant to the cause of L-112's stranding. For the vitamin A level, only retinol levels were measured as post-mortem degradation can rapidly deteriorate levels.

Overall, ranked concentrations of POPs in the blubber of L-112 (Table 3) were $\sum$DDTs > $\sum$PCBs >> $\sum$ chlordanes > $\sum$PBDEs > HCB > $\sum$HCHs. On a lipid basis, concentrations of HCB in the 0 to 2 cm depth (most similar to a biopsy sample) of blubber of L-112 (1,200 ng/g lw) were in the same range as those from other juvenile SRKWs (range 440-2,000 ng/g, lw). Concentrations of PCBs in the blubber of L-112 (39,000 ng/g lw) were also comparable (range 17,000-60,000 ng/g lw). PCB concentrations were also comparable with the mean of $\sum$PCBs in biopsy samples collected between 1993 and 1996 from adult male northern resident killer whales, as reported by Ross et al. (2000) (a mean of 37,400 ng/g lw, n=8).

Total DDTs measured in the 0 to 2 cm blubber layer of L-112 (Table 3) were at the high end of the range for - juvenile SRKWs (60,000 ng/g lw; range 20,000 – 93,000 ng/g lw). The $\sum$DDT/$\sum$PCB ratio in blubber from L-112 (~1.6) was similar to those from other juveniles from K and L Pods (range 1.2 to 1.8) and higher than those from juveniles from J Pod (range 0.6 to 0.7), reflecting differences in seasonal movements between these groups.

Total PBDEs measured in the 0 to 2 cm blubber layer of L-112 (Table 3) were on the lower end of the range compared to those of other juvenile SRKWs (4,600 ng/g lw; range 3,500 – 15,000 ng/g lw) but more than an order of magnitude higher than PBDE concentrations measured in biopsies of male northern residents reported by Rayne et al. (2004) (a mean of 203 ng/g lw, n=9).

Although PBDEs are an emerging concern in marine and terrestrial biota, few recent measurements have been made in killer whales or other species. Most published measurements have been made on archived samples, as was true of the samples reported in Rayne et al. (2004), which were collected between 1993 and 1996. Because PBDEs are still used in North America, environmental PBDE levels may continue to rise. Because of this and biological factors such as maternal offloading of contaminants during gestation and lactation, juvenile killer whales may be particularly at risk. Higher average levels of PBDEs compared to adults have been measured recently in juvenile SRKWs (Krahn et al. 2009), as well as insular Hawaiian Island false killer whales (Ylitalo et al. 2009).

The levels of persistent organic pollutants in the blubber of L-112 exceed the thresholds for some biological effects, particularly immunosuppression and thyroid hormone and retinol disruption (AMAP 2004). The levels also exceed thresholds associated with reproductive success (AMAP 2004). One caution is that these thresholds are determined mostly from studies with captive animals (e.g., harbor seal, mink), and how these thresholds compare to effects thresholds in wild populations of other species is somewhat speculative. There is no evidence in the literature to suggest that the levels of POPs found in the blubber of L-112 would make an animal more susceptible to death by trauma.

This multi-disciplinary investigation could not determine the source of the blunt trauma despite gathering and evaluating all available information on the whales, the environment, and human activities. We evaluated the sighting history of the whales to provide insight into the circumstances of the stranding. Autonomous passive acoustic recorders off the coasts of Washington, Oregon, and California indicated that the main group of L Pod, possibly including L-112, was off California in late January, heading north, and possibly off Westport, Washington in the first week of February and detected near Newport, Oregon after the stranding. Members of the L4s and L-112 were not seen with the group of K and L Pod whales observed in Discovery Bay on February 7. The information suggests that L-112 was more likely to have been at sea off southern Washington or Oregon at the time of her death rather than in the inland waters of the Salish Sea.

There was no indication of a mass stranding event underway when L-112 was discovered and no additional marine mammals, dead fish, or birds were recovered or reported in the area at the time. A major source of trauma from sonar, explosives, or a seismic event would likely have affected multiple individuals traveling together as killer whales are known to do. All other members of L-112's family group were sighted following L-112's stranding. No other members of the L4 sub-group were reported missing, injured, or stranded between the time of the L-112 stranding and the summer of 2012. This observation leads us to believe that the trauma suffered by L-112 was likely borne individually and was not an event that covered a large area or that directly impacted the young whale's most likely traveling companions in the L4 sub-group. For these reasons, we do not believe that L-112 succumbed to blast injuries or exposure to other high intensity sound.

The flow models and drift card studies indicate that current conditions off the Long Beach Peninsula are largely influenced by eddies created by flows from the mouth of the Columbia River. In the days prior to the stranding, eddies would have flowed northward under the influence of the prevailing wind and currents, allowing floating debris trapped in eddies to be deposited on Long Beach. Floating debris arriving from the open sea to the west or north of Long Beach would have been carried northward by the current to be deposited elsewhere on the Washington or British Columbia coasts. Because of prevailing currents and eddies it is unlikely that L-112 died in Canadian waters or the Strait of Juan de Fuca and drifted south, but instead likely died in the Columbia River plume or farther to the south along the coast of Oregon. Given the state of decomposition at the time of stranding the body was either carried by eddies for several days or may have drifted a substantial distance from the south before being trapped by the eddies and cast ashore on the Long Beach Peninsula.

Sea surface temperatures along the central Oregon to southern Washington coast in early February, where and when L-112 likely died, did not appear to vary markedly from the same time frame the previous or following year. Temperature does not appear to be a contributing factor to this stranding event.

As a result of inquiries for information on military exercises we learned that no U.S. or Canadian military activities involving sonar or explosives, except those reported from Canada and the Strait of Juan de Fuca, were undertaken off the coast of Oregon or Washington where L-112 appears most likely to have been at the estimated time of her death. Similarly, there were no in-water construction or seismic activities using explosives either permitted or reported in the area of the stranding, nor were any explosive events detected on the hydrophones deployed near Westport, Washington, or Newport, Oregon at the time. The CT results showed no evidence of bone fractures or damage to the middle or inner ear bones. These results do not conflict with gross observations and the proposed cause of acute or peracute death by blunt force trauma; however, blast- or seismic-related injuries cannot be entirely discounted. We acknowledge that post-mortem decomposition may have obscured some lesions and hindered mass-spectroscopy gas analysis.

Little information is available on the response of odontocetes to earthquakes. In general, earthquakes are low frequency, under 100 Hz, which is outside of the hearing range of killer whales, although they may cause disorientation for species with low frequency hearing sensitivity. Killer whales are considered to have mid-frequency hearing and ranges from 1 to at least 120 kHz, but tend to be most sensitive in the range of 18 to 42 kHz (Szymanski et al. 1999). The investigative team concluded that small magnitude earthquakes were unlikely to have caused the traumatic injuries noted during the L-112 post-mortem exam. We ruled out an earthquake as a causative factor because the earthquakes recorded off southern Oregon in early February were low magnitude (≤ 4), and a larger magnitude quake (4.8) off Vancouver Island was to the north and therefore “downwind” from the stranding. There was no evidence of wide-spread damage or disturbance of wildlife in the area of the stranding.

There was no gross indication of fisheries interaction, such as external markings from nets, hooks, or lines, and there were no reports of interactions from the fishing community. No vessel strikes were reported; however, we couldn’t rule out a vessel strike. Gaydos and Raverty (2010) summarized killer whale strandings from 2005 to 2010, and human interactions, including fishery interactions and vessel strikes, were implicated in the deaths of three whales and possibly two others:

- A female calf died of complications secondary to fishing interaction (Glacier Bay, Alaska, 2005)
- A juvenile male was drawn through the propeller of a tug boat (Gold River, British Columbia, Southern Resident L-98, 2006)

- An adult female presented with hemothorax and a subcutaneous hematoma in the neck (Prince Rupert / Port Simpson, British Columbia, Northern Resident C-21, 2006)
- An adult female featured a subcutaneous abscess and possible vertebral fractures (Humboldt County, California, Transient N-18; 2005)
- An adult female was suspected to have been hit by a large propeller (Westport, Washington, Transient T-086, 2007)

The presentation of L-112 is not consistent with other killer whale vessel strike cases, which included more definitive clues, such as propeller marks or broken bones.

Inter-species and intra-species aggression is documented for a variety of odontocete cetaceans including bottlenose dolphins (*Tursiops truncatus*) (Patterson et al. 1998; Robinson 2013; Kaplan 2009), Pacific white sided dolphins (*Lagenorhynchus obliquidens*) (Baird 1998), harbor porpoises (*Phocoena phocoena*) (Jepson et al. 1998; Barnett et al. 2009; Patterson et al. 1998), Atlantic spotted dolphins (*Stenella frontalis*) (Herzing et al. 200;, Herzing et al. 1997), costeros (*Sotalia guianensis*) (Wedekin et al. 2004), Commerson's dolphins (*Cephalorhynchus commersonii*) (Coscarella et al. 2010), and killer whales (Ford and Ellis 1999; Jefferson et al. 1991). In many cases, the targets of aggression are calves or neonates. The primary signs of injury reported from aggressive attacks are rake marks, musculoskeletal and/or intra tissue trauma (bruising, tearing) attributed to ramming and sometimes death. Contrary to the cases reported in the literature, L-112 was a juvenile animal (older and larger than a calf or neonate), and the examiners did not document tooth rake marks associated with the signs of hemorrhage they observed during the gross examination. Nevertheless, we cannot rule out the possibility that L-112 suffered injuries from an aggressive attack, such as ramming, by a larger animal.

While the extensive evaluations were all consistent with blunt force trauma from a collision or blow being the cause of death, the exact type or source of the traumatic injuries (what struck the animal) remains unknown. Although we could not identify a definitive source of the trauma or cause of death, the thorough investigation provides a unique look into the threats facing SRKWs. A recent review of killer whale strandings in the North Pacific Ocean (Barbieri et al. 2013) highlights the value of stranding investigations as an integral component of a comprehensive population health assessment program, as they yield data on mortality, life history, and threats to conservation. The measurement, prey, and contaminants data from this investigation of L-112 are valuable and provide additional data for ongoing studies and efforts to recover endangered SRKWs.

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FIGURES

A. B.

Figure 1. A. Female killer whale stranded on Long Beach February 11, 2012. Laying on right side. Note red discoloration of the skin on left side of head near gape and “rub” posterior to flipper. B. Note red discoloration on ventral surface of head, neck, and anterior chest.

Figure 2. Detailed photograph of extensive red discoloration of the skin on left side of head.

Figure 3. Whale was moved to Cape Disappointment State Park for necropsy.

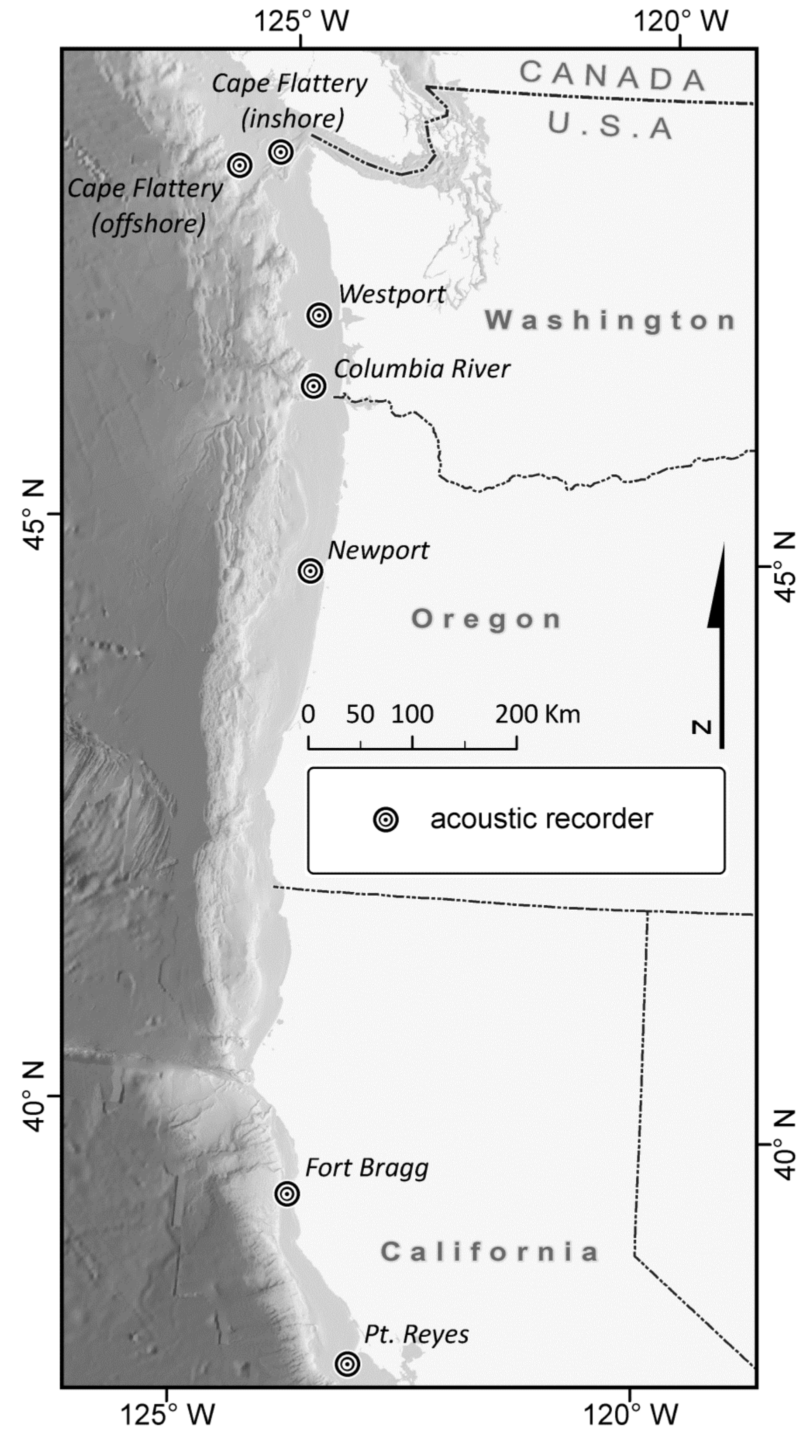

Figure 4. Location of acoustic recorders deployed by NWFSC in 2011 and 2012.

A. B.

Figure 5. A. View of bruising on left side of head. B. Extensive congestion, possibly hypostasis, is apparent along right side of head and thoracic wall.

A. B.

Figure 6. A. Extensive bruising on ventral aspect of lower jaw and chest. Note however that the discoloration appears more apparent here than in previous photos and suggests hypostasis, rather than bruising. B. Close-up of ventral bruising on lower jaw. See also Figure 2.

Figure 7. Subcutaneous swelling around the genital slit with partial evagination of vagina.

A. B.

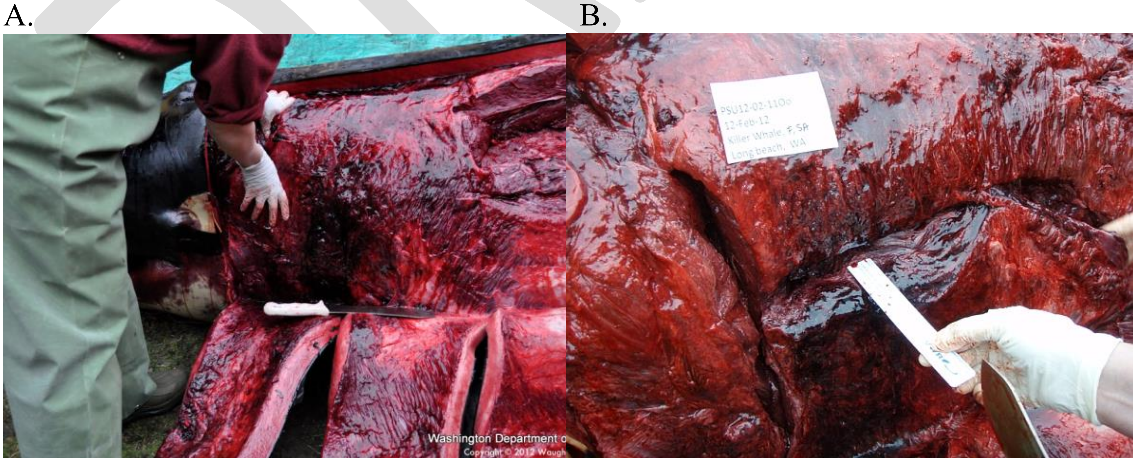

Figure 8. A. Hemorrhage in soft tissues extending under the scapula on the left side.
B. Hemorrhage extending deeper in the chest wall musculature.

A. B.

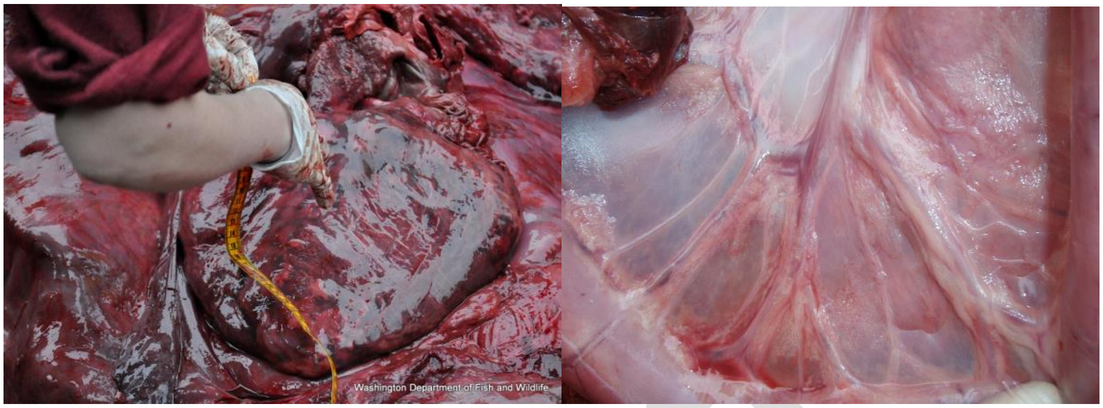

Figure 9. A. Red serous fluid in heart lining. B. Blood was absent from arteries and veins as seen here in the mesenteries, although there were clear, well delineated intravascular spaces (presumptive gas emboli). Gases were consistent with decomposition.

A. B.

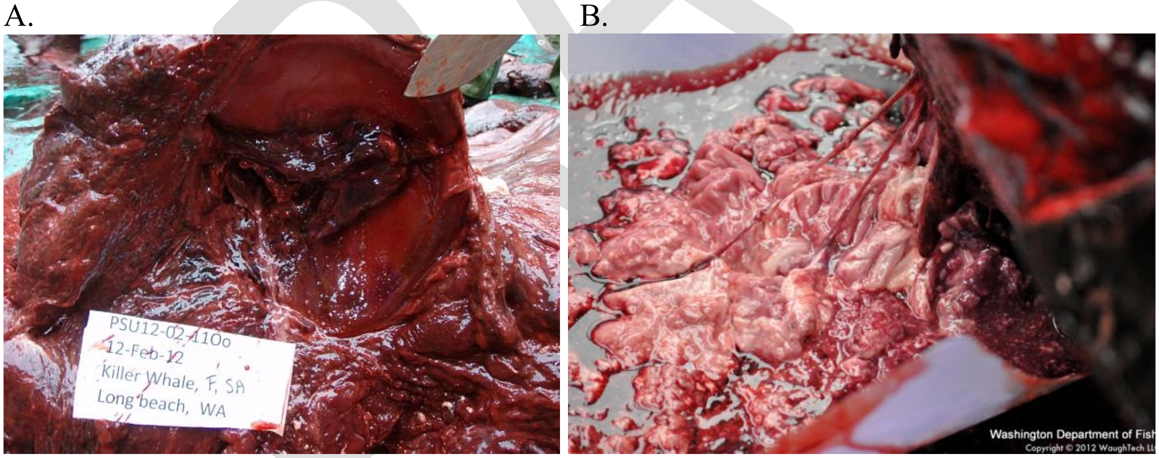

Figure 10. A. Tissues of the calvaria were dark red and filled with red serous fluid surrounding. B. Copious dark red serous fluid and brain fragments flowed from the foramen magnum.

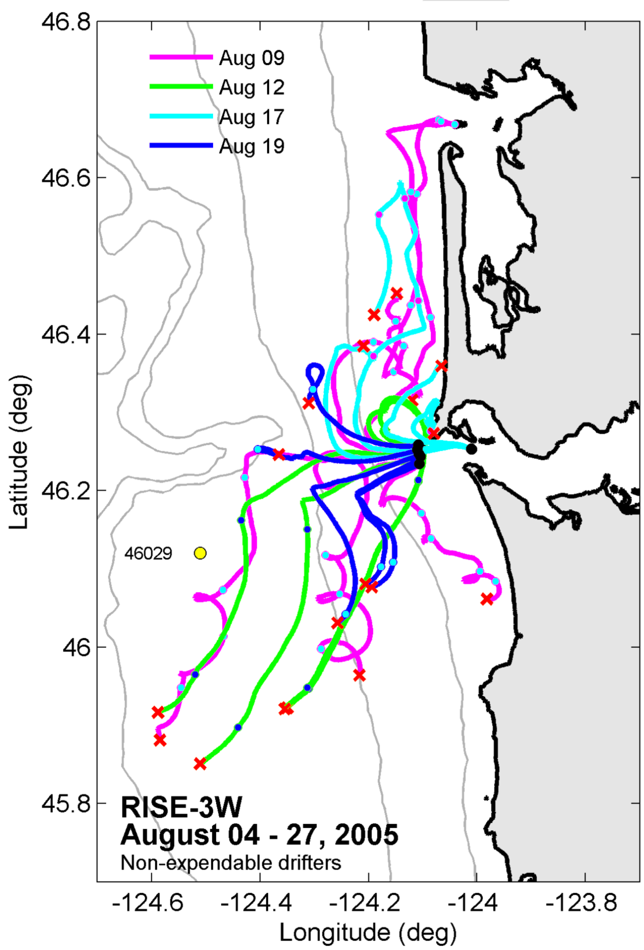

Figure 11. Patterns of drift for surface drifters deployed by the University of Washington off the mouth of the Columbia River from August 9-19, 2005.

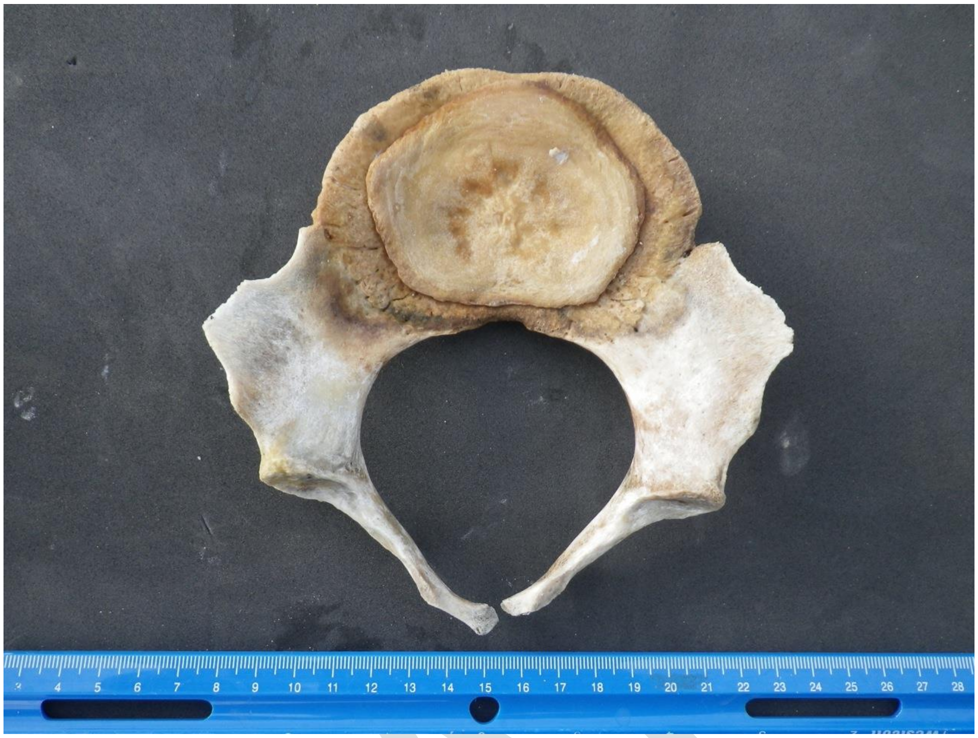

Figure 12. The dorsal aspect of cervical vertebra C7 showing incomplete fusion of the lamina and incomplete formation of the spinous process.

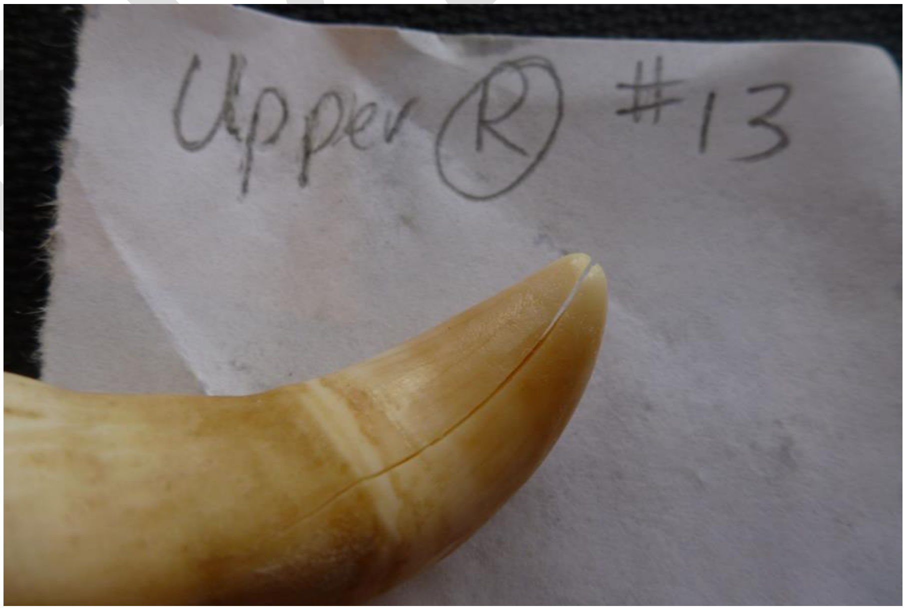

Figure 13. Linear vertical fracture in the upper right jaw tooth #13, the defect extended to the pulp cavity.

LIST OF APPENDICES